

## Prevalence of *Cryptosporidium* oocysts in cattle from Southern Ghana

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### ABSTRACT

The prevalence of *Cryptosporidium* species oocysts in 241 cattle from two agroecological zones in Southern Ghana was investigated in this study. *Cryptosporidium* oocysts were demonstrated in fecal samples using sodium chloride centrifugal floatation, followed by the modified Ziehl-Neelsen acid-fast staining technique. Overall, 29.0% of the cattle were positive for *Cryptosporidium* oocysts. Prevalence was significantly higher ( $P < 0.001$ ) among cattle from the forest-savannah transition zone (50.6%) compared to the coastal savannah zone (18.9%). *Cryptosporidium* oocysts were detected in both males (28.4%) and females (31.8%) with no significant difference ( $P > 0.05$ ). Age group prevalence was 25.8%, 33.3% and 27.6% in calves ( $\leq 10$  months old), young cattle (11 to 24 months old) and adults ( $> 24$  months old) respectively ( $p > 0.05$ ). *Cryptosporidium* oocysts were detected in 23.1% of 39 diarrheic and 30.2% of 202 non-diarrheic stools ( $P > 0.05$ ). The majority of infected cattle were asymptomatic. These findings suggest that cattle may play a role in the zoonotic transmission of *Cryptosporidium* oocysts to humans and other animals in Ghana.

**Key words:** *Cryptosporidium* spp., oocysts, prevalence, diarrhoea, cattle, Ghana

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### Introduction

*Cryptosporidium* is an intracellular coccidian parasite, recognized worldwide to cause diarrhoea in several vertebrates (FAYER and UNGAR, 1986). The parasite was first described in mice in 1907 (TYZZER, 1907). It was not recognized as an important pathogen until the 1970's, when it was linked to chronic diarrhoea in an 8-month-old heifer (PANCIERA et al., 1971) and later to humans (MEISEL et al., 1976; NIME et al., 1976). *Cryptosporidium* has since become an interesting emerging infection owing to its opportunistic behaviour in AIDS patients (PETERSON, 1992; NAVIN and HARDY, 1987; McGOWAN et al., 1993) and children (VALENTINER-BRANTH et al., 2003; MOR and TZIPORI, 2008).

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*Cryptosporidium* is also known to cause varying degrees of diarrhoea in cattle, the main clinical manifestations include diarrhoea, abdominal pain, anorexia and depression (FAYER, 1998a; DE GRAAF et al., 1999; FAYER et al., 2000). Consequently, there are reports of zoonotic transmission of *Cryptosporidium* from calves to humans (CASEMORE, 1990; CASEMORE et al., 1997; MAHDI and ALI, 2002).

In Ghana, studies on *Cryptosporidium* have largely involved the detection of oocysts in fecal samples using the acid-fast modified Ziehl-Neelsen technique, in children (ADJEI et al., 2004), HIV/AIDS patients (ADJEI et al., 2003), food vendors (AYEH-KUMI et al., 2009) and drinking water (KWAKYE-NUAKO et al., 2007). These investigations, in addition to the detection of *Cryptosporidium* in paediatric diarrhoeic samples using real-time PCR by OPINTAN et al. (2010), were all carried out in Southern Ghana. Available reports therefore signify the public health importance of *Cryptosporidium* in Ghana and the need for investigations in animals as well.

Furthermore, in Ghana cattle are an integral part of the livestock industry for the purpose of mainly beef and in some cases milk production (OKANTA, 1992). The extensive system is the main cattle production system practiced, based mainly on extensive grazing by smallholder herds. In addition, commercial cattle farming and state institution farms practice varying levels of management systems that are higher than the extensive system. Here, the cattle may graze on sown pastures as well as natural pastures. (OPPONG-ANANE et al., 2010). These management systems bring cattle into close association with other animals and humans in this country, with water bodies (e.g. dams, rivers and streams) as the main point of interaction. This study therefore sought to determine the prevalence of *Cryptosporidium* spp. oocysts in cattle from Southern Ghana.

### Materials and methods

**Study area.** The study was conducted at two selected farms of a state institution, located at Pokuase (latitude 5° 41' N, longitude 0° 17' W) in the forest - coastal savannah transition zone and Fafraha (latitude 5° 43' N, longitude 0° 08' W) in the coastal savannah zone of Southern Ghana. The Pokuase farm's mean monthly temperature ranged from 23.3 °C to 24.7 °C and rainfall from 12.6 mm to 204.6 mm. The drier Fafraha farm, however, recorded mean monthly temperatures from 25.7 °C to 29.2 °C and rainfall from 0 to 205.7mm. Cattle were kept in kraals and grazed on natural pastures, substituted with cultivated pastures. The herd obtained drinking water from either a dam or stream within the rangeland. Climatic data obtained from the Ghana Meteorological Services Department in Accra were recorded from January to December 2009 on the farms.

**Fecal sample collection.** Fresh fecal samples were randomly collected directly from the rectums of 241 cattle in a cross sectional study between June and July 2009. The samples were placed in suitable leak-proof plastic containers, tightly closed, labeled and

transported to the laboratory for examination. Details of location, age group and sex of animals were recorded.

*Parasitological techniques.* The fecal samples were examined macroscopically for their consistency as watery, loose, soft or formed. Oocysts were concentrated by sodium chloride centrifugal floatation of fecal samples, after which excess salt was washed with water to avoid deterioration of oocyst morphology. *Cryptosporidium spp.* was detected using the Modified Ziehl-Neelsen acid-fast staining technique (CASEMORE et al., 1985; ANONYM., 2008). Samples that could not be examined immediately were then stored at 4°C after adding 2.5% potassium dichromate to control the growth of microorganisms. Microscopic identification was done by a light microscope under 40x and then 100x objective oil-immersion lens. Morphometry was performed with the aid of a micrometer.

*Statistical analysis.* Results were analyzed using SPSS 15.00 for Windows (SPSS, 2006) to perform chi-squared tests, which were used to compare the differences in prevalence between groups at 0.05 level of significance. T-tests were also performed to compare the means of climatic data at 0.05 level of significance.

## Results

*Climatic data of farm locations.* The mean monthly temperature, rainfall and number of rain days for the two locations are shown in Fig. 1, 2 and 3. The mean annual temperature at the Fafraha farm ( $27.7 \pm 0.4$  °C) was significantly higher ( $P < 0.001$ ), compared to the Pokuase farm ( $24.2 \pm 0.1$  °C). Conversely, there was no significant difference ( $P > 0.05$ ) between the mean annual rainfalls ( $61.7 \pm 15.5$ mm and  $54.7 \pm 18.2$ mm) as well as number of rain days ( $6.1 \pm 1.1$  days and  $4.6 \pm 1.2$  days) for Pokuase and Fafraha farms, respectively.

During the period of fecal sampling (June and July), climatic data indicated the following means: rainfall ( $205.2 \pm 0.5$  and  $155.7 \pm 50.0$ ;  $P < 0.05$ ), number of rain days ( $10 \pm 2$  and  $12 \pm 5$ ;  $P > 0.05$ ) and temperature ( $24.2 \pm 0.4$ °C and  $26.5 \pm 0.6$  °C;  $P > 0.05$ ) at Pokuase and Fafraha farms respectively. The major rainy season peaked in June during the year, at both locations.

*Prevalence of Cryptosporidium oocysts in fecal samples of cattle.* An overall prevalence of 29.0% (70/241) for *Cryptosporidium* oocyst infection was observed among the cattle. *Cryptosporidium* oocysts were detected in all the age groups as shown in Table 1. A prevalence of 25.8% (16/62), 33.3% (27/81) and 27.6% (27/98) oocyst positives were observed in calves ( $\leq 10$  months old), heifers and young bulls (11 to 24 months old) and adults ( $> 2$  years old) respectively. There was no significant difference in the age group prevalence ( $P > 0.05$ ).

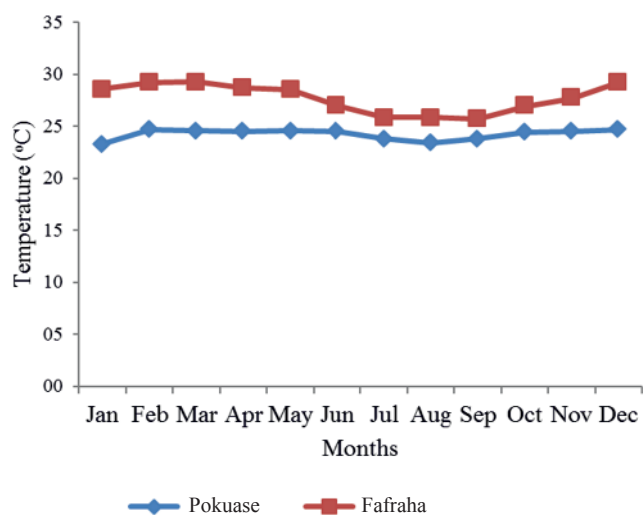


Fig. 1. Mean monthly temperature

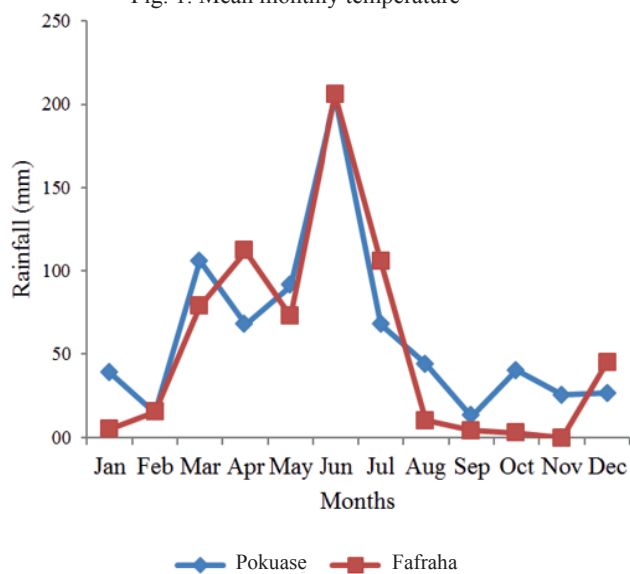


Fig. 2. Mean monthly rainfall

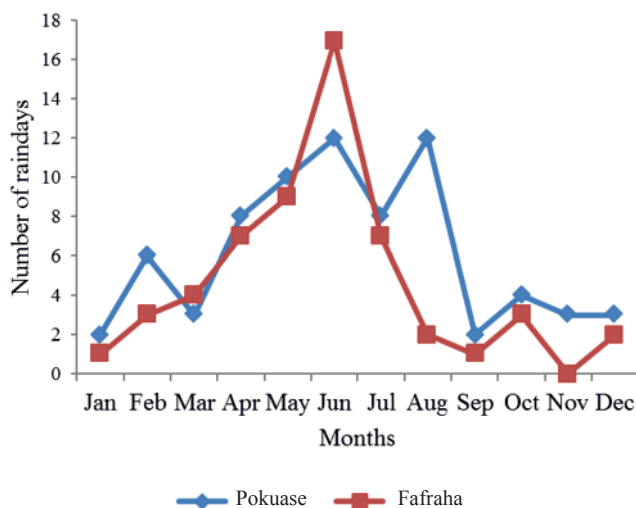


Fig. 3. Mean monthly number of rain days

Table 1. Prevalence of *Cryptosporidium* oocyst with age group and diarrheal status of cattle

Age group	Number tested	Number positive (%)	Diarrheic		Non-Diarrheic	
			Number tested	Number positive (%)	Number tested	Number positive (%)
≤ 10 months	62	16 (25.8)	9	1 (11.1)	53	15 (28.3)
11-24 months	81	27(33.3)	12	3 (25.0)	69	24 (34.8)
>24 months	98	27(27.6)	18	5 (27.8)	80	22 (27.5)
Total	241	70 (29.0)	39	9(23.1)	202	61 (30.2)

Age group prevalence:  $X^2 = 1.144$  ( $P > 0.05$ ); Diarrheal prevalence:  $X^2 = 0.546$  ( $P > 0.05$ )

An examination of the consistency of the fecal samples showed that 16.2% (39/241) of samples were diarrheic. Of these, 23.1% (9/39) tested positive for *Cryptosporidium* oocysts. The remaining 83.8% (202/241) were non-diarrheic, with 30.2% (61/202) of these testing positive (Table 1). There was no significant difference ( $P > 0.05$ ) between the number of diarrheic samples with *Cryptosporidium* oocysts compared to the non-diarrheic samples. Among the calves, only 1.1% (1/9) of the diarrheic samples were positive, whilst 28.3% (15/53) of the non-diarrheic samples tested positive ( $P > 0.05$ ). A similar trend was observed among heifers and young bulls, and the adults, as summarized

in Table 1. Altogether, 87.1% (61/70) of the cattle with *Cryptosporidium* infection were non-diarrheic.

Across the agro-ecological zones (farms), *Cryptosporidium* spp. oocyst infection was significantly higher ( $p < 0.001$ ) among the herd at Pokuase, in the transitional zone, (50.6%; 39/77) than Fafraha, in the coastal savannah zone (18.9%; 31/164), as shown in Table 2.

Table 2. Prevalence of *Cryptosporidium* oocysts in cattle with agroecological zones and sexes of cattle

	Number tested	Number positive	Prevalence (%)	Chi-square value	P-value
Agro-ecological zone					
Transition Zone (Pokuase)	77	39	50.6	25.625	<0.001
Coastal Savannah Zone (Fafraha)	164	31	18.9		
Sex					
Female	197	56	28.4	0.201	0.654
Male	44	14	31.8		

Table 2 also shows no significant difference ( $P < 0.05$ ) between 28.4% (56/197) females and 31.8% (14/44) males positive for *Cryptosporidium* oocyst.

### Discussion

It is evident from these data shown that, *Cryptosporidium* spp. oocysts are present in cattle from the two farms in Southern Ghana. The overall prevalence (29.0%) in cattle observed in this study is within the range of prevalence (2.5% to 37.0%) reported in humans in Ghana (ADJEI et al., 2003; ADJEI et al., 2004; AYEK-KUMI et al., 2009). This prevalence is also consistent with reports of cattle from other African countries: 19.7% in Tanzania (SWAI et al., 2007) and 23.4% in Nigeria (AYINMODE and FAGBEMI, 2010). Our finding affirms the veterinary and public health importance of *Cryptosporidium* in Ghana, as in other countries.

The results showed a prevalence of 25.8% among calves. This is in accordance with reports elsewhere: 19.2% in Zambia (GEURDEN et al., 2006), 35% in Tanzania (SWAI and SCHOONMAN, 2010) and 35.5% in Norway (SANTIN et al., 2004) although the age categories varied. Our findings showed no significant difference ( $P > 0.05$ ) in the rate of infection among the calves, heifers and young bulls, and adults. This report is contrary to a previous report that indicated that the prevalence of cryptosporidiosis is higher in calves than in adult cattle (CURRENT and GARCIA, 1991).

In addition, *Cryptosporidium*, an important agent in the etiology of neonatal diarrhea syndrome in calves, lambs and kids, invariably causes considerable direct and indirect economic losses (DE GRAAF et al., 1999). In this study however, calves with diarrheic stools were not more positive for *Cryptosporidium* spp. compared to those without diarrheic stools ( $P>0.05$ ). Diarrheal symptoms in calves could be ascribed to other factors other than age. To study the role of *Cryptosporidium* in diarrhea among young animals in Ghana, future studies should involve larger populations of diarrheic calves.

In the present study, the majority (87.1%) of the *Cryptosporidium* infected cattle were non-diarrheic, which indicated that mostly asymptomatic animals shed *Cryptosporidium* oocysts. These animals may act as reservoirs of the protozoan parasite and discharge oocysts into the environment to infect other animals and humans.

Data from this study showed a significantly higher ( $P<0.001$ ) prevalence of *Cryptosporidium* infection in cattle from the transitional zone (50.6%) as compared to the drier coastal savannah zone (18.9%). This could be attributed to the variation in meteorological conditions in the various locations of the farms. Although there was no statistically significant difference in mean annual rainfall and number of rain days, the mean annual temperature on Fafraha farm (coastal savannah zone) was higher ( $<0.001$ ) than Pokuase farm (transitional zone). This information therefore supports reports that oocysts can remain viable for many months in the environment, but higher temperatures and desiccation results in more rapid loss of viability, and thereby decreasing infectivity (FAYER, 1994; FAYER et al., 1998b).

According to JAGAI et al. (2009), the seasonal patterns in cryptosporidiosis infection can be substantially affected by seasonal variations in exposure levels associated with water quality and access to water, as well as wildlife and agricultural activities, which in turn can be affected by meteorological characteristics, such as temperature and precipitation. The overall prevalence observed in this study could have therefore been affected by the peak of the rainy season, when fecal samples were collected.

In addition, the quality of drinking water available to the animals may have contributed to the variation in prevalence among cattle from the different agro-ecological zones. Drinking water for the herds at Fafraha farm was an enclosed dam, while Pokuase farm had an open stream flowing from the town, more prone to contamination. Further investigation into the seasonal variation, water quality and oocyst infection level could provide more information for a better understanding of the problem.

The results in this study showed no significant difference ( $P>0.05$ ) between the rate of infection between female and male cattle as indicated by others (AYINMODE and FAGBEMI, 2010; MALLINATH et al., 2009). These authors attributed their disparity to the usual practice of having a higher female to male ratio in a herd and the retention of

female animals for breeding and milk production. The reason for the current observation is however not obvious.

The present study showed that, *Cryptosporidium* oocysts are present in cattle in southern Ghana, with a higher prevalence in the transition zone compared to the drier coastal savannah zone, amidst other climatic and environmental factors. The overall prevalence was consistent with those of similar studies in other countries, however, infection was linked to neither diarrheic stools nor higher prevalence in calves. Our findings showed that most of the cattle shedding oocysts were asymptomatic and could serve as reservoirs of *Cryptosporidium* oocysts.

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**SQUIRE, S. A., J. BEYUO, H. AMAFU-DEY: Prevalencija oocista kriptosporidija u goveda u Južnoj Gani. Vet. arhiv 83, 497-507, 2013.**

**SAŽETAK**

Istraživana je prevalencija oocista vrsta roda *Cryptosporidium* u 241 govedu na dvama agroekološkim područjima u Južnoj Gani. Oociste kriptosporidija bile su dokazane u uzorcima fecesa metodom flotacije u fiziološkoj otopini te naknadnim bojenjem po preinačenom Ziehl-Neelsenovom postupku acidorezistentnog bojenja. Oko 29,0% goveda bilo je pozitivno na oociste kriptosporidija. Prevalencija je bila značajno veća ( $P < 0,001$ ) u goveda na prijelazu sa šumskog na stepsko (livadno) područje (50,6%) u usporedbi s priobalnim stepskim područjem (18,9%). Oociste kriptosporidija bile su bez značajnih razlika ( $P > 0,05$ ) dokazane u mužjaka (28,4%) i ženki (31,8%). Prevalencija u teladi (u dobi  $\leq 10$  mjeseci) iznosila je 25,8%, u junadi (od 11 do 24 mjeseci) bila je 33,3%, a u odraslih ( $> 24$  mjeseci) 27,6% ( $P > 0,05$ ). Oociste kriptosporidija bile su dokazane u 23,1% od 39 uzoraka proljeva i 30,2% od 202 uzorka normalno formiranog fecesa ( $P > 0,05$ ). Većina zaraženih goveda nije pokazivala nikakve znakove bolesti. Ovi nalazi upućuju na zaključak da govedo može imati ulogu u zoonotskom prijenosu oocista kriptosporidija na čovjeka i neke vrste životinja u Gani.

**Ključne riječi:** *Cryptosporidium* spp., oociste, prevalencija, proljev, govedo, Gana

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